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MODELS OF THE TUMOUR SPHEROID RESPONSE TO RADIATION: IDENTIFIABILITY ANALYSIS

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Abstract

Two spatially uniform models of tumour growth after a single instantaneous radiative treatment are presented in this paper. The two ordinary differential equations models presented may be obtained from two equivalent partial derivative equations models, by integration with respect to the radial distance. The main purpose of the paper is to study their identifiability properties. In fact, a preliminary condition, that is necessary to verify before performing the parameter identification, is the global identifiability of a model. A detailed study of the identifiability properties of the two models is done, pointing out that the first one, the basic model, is only locally identifiable, whereas the second one, the model with subcompartments, is globally identifiable, provided that the responses to two different radiation doses are available.

Keywords: Tumour spheroids models; Radiotherapy; Local and global identifiability.

1 Introduction

The mathematical literature on solid tumour growth is very wide. Looking through it, this evolution line can be recognized: the earliest models were focused on avascular tumour growth; then models of angiogenesis were developed; more recently, models of vascular tumour growth are starting to emerge [1].

With reference to mathematical models of avascular tumour growth we can underline the presence of two different kinds of models: the spatially uniform models and the spatially structured models.

The first class of models concerns with models in which details of the spatial structure of the tumour are neglected and the attention is focused, for instance, on the tumour overall volume or on the total number of cells present within the tumour itself. The resulting models are formulated as systems of ordinary differential equations (ODE models) and have been widely used by clinicians to estimate kinetics parameters associated to tumour growth *in vivo* and *in vitro* and to asses the efficiency of different therapeutic strategies [1].

On the other hand, the second class concerns with models in which the spatial coordinates are taken into account, in order to investigate the role of rate limiting, diffusible *growth factors* on the tumour development. When the geometry of the tumour is simple (for instance the spherical geometry of tumoral spheroids *in vitro* or of small metastasis *in vivo*, or the cylindrical geometry of the tumoral cords) it is possible to take the spatial structure into account only considering one spatial coordinate (one dimensional growth). These models are formulated as systems of partial derivative equations (PDE models) and typically comprise reaction-diffusion equations for the growth factors and an integro-differential equation for the tumour radius, in the case of spherical or cylindrical symmetry. The reaction-diffusion equations are necessary in such models to take into account the diffusion through the tumour of internal (produced by cells) or external (externally supplied) chemical agents and to predict their concentration variations. The chemicals of interest may be, for instance, glucose or oxygen, that promote the cell division, or chemotherapeutic drugs, tumour necrosis factors and products of cell degradation, that promote the cell death [1].

In this paper two spatially uniform models of tumour growth, after a single instantaneous radiative treatment are presented, with the main purpose of studying their identifiability properties. These models come from the integration with respect to the spatial coordinate of the partial derivative equations of two spatially structured models [2], [3], [4], when it is possible to neglect the distribution of oxygen concentration inside the tumour. In fact, the oxygen concentration is generally very important in such models because it influences the radiosensitivity of cells [5] and it determines the cell death when its level is too low. Nevertheless, when the tumoral spheroid, during all its growth, remains smaller than a critical dimension at which an internal necrotic region starts to develop, then it can be assumed that:

1. the oxygen concentration is higher than the minimum value necessary to the cell life
2. the initial distribution of oxygen inside the spheroid is sufficiently uniform to be assumed constant

Thus, in view of 1. the cell death for insufficient oxygenation can be neglected and the radiation is the only cause of death. Moreover for 2. it can be assumed that the radiosensitivity coefficients are constant for all the tumoral cells inside the spheroid. With these two assumptions, the two ODE models presented in this paper are completely equivalent to the original PDE models presented in [2], and they may be obtained from the latter by integration with respect to the radial distance, as mentioned above.

2 ODE mathematical modelling of the tumour spheroid response to radiation

2.1 The linear quadratic model for the radiation action

Radiation produces a variety of lesions in a cell [6]. These lesions induce a lethal damage in a fraction of cells, that loses the capacity of continuous proliferation and will die at a subsequent time (clonogenically dead cells). Thus, after irradiation, the living tumour cell population will be composed by a subpopulation of viable cells and a subpopulation of live but lethally damaged, clonogenically dead cells. The death of lethally damaged cells may occur by premitotic apoptosis or after one or more cell divisions (postmitotic apoptosis).

The main pathways of lethal damage production are the direct action of radiation that produces unreparable damages and the binary misrepair of double-strand breaks (DSB) of DNA. In the case of impulsive irradiations, both the direct action and the effect of binary misrepair will be considered instantaneous and described by a non linear relation named linear-quadratic (LQ) model [7]. Denoting by δ the surviving fraction of cells after a single impulsive irradiation, the LQ dose-response relation has the form:

$$\delta = e^{[-\alpha d - \beta d^2]} \,, \tag{1}$$

where d is the dose, and α and β the radiosensitivity parameters, related, respectively, to the direct action of radiation and to the binary misrepair of DSBs. The equation (1), as shown in the following, will be used in the models presented in this paper to initialize the state vector, taking the effect of the radiation into account.

2.2 The basic dynamical model

Although quiescent cells have been evidenced in tumour spheroids [8], [9] for simplicity we will assume that all viable cells proliferate with the same rate and this assumption is reasonable because the models are formulated under the assumption of 'small spheroids', where the oxygen level is sufficiently high and uniform. So in a spheroid we will distinguish: viable cells, lethally damaged cells and dead cells. The model variables are the total volumes of the three types of cells inside the spheroid and they are functions of t. So we denote with $V(t)$, $V_D(t)$ and $V_N(t)$, the volumes of viable cells, lethally damaged cells and dead cells, respectively obtained by integration, with respect to the radial coordinate, of the local volume fractions of the model in [2].

The following main assumptions are essential for the spatial integration of the original PDE equations [2]: the growth of the tumour spheroid never goes over a critical dimension, at which the internal necrosis starts to occur (this dimension depends on the external oxygen concentration and it has been found to be $200 \div 300\mu m$ [2] in standard *in vitro* conditions, with an oxygen concentration of $0.28mM$), and the initial spheroid dimension is 'sufficiently' smaller than this critical dimension.

Under these hypothesis, by integrating the PDE equations presented in [2], [4] the following basic model can be obtained:

$$\begin{cases} \dot{V}(t) = \chi V(t) \\ \dot{V}_D(t) = (\chi_D - \mu_D) V_D(t) \\ \dot{V}_N(t) = \mu_D V_D(t) - \mu_N V_N(t) \end{cases}, \tag{2}$$

where with χ and χ_D we denote the constant proliferation rates, respectively, of the viable cells and of the lethally damaged cells (that we suppose to progress across the cell cycle and to divide until they die), with μ_D and μ_N, respectively, the death rate of the lethally damaged cells and the degradation rate of the dead cells. All these dynamic parameters are positive and, since the lethally damaged cells eventually die, it is necessary to assume that $\mu_D > \chi_D$. The output of the model is the total volume of the spheroid, obtained by the sum of the state variables:

$$y(t) = V(t) + V_D(t) + V_N(t)\,. \tag{3}$$

Without loss of generality, cells are assumed to occupy all the volume of the spheroid.

Considering only impulsive irradiations, the initial conditions for the basic model, according to (1), are:

$$\begin{cases} V(0^+) = e^{[-\alpha d - \beta d^2]} V(0^-) \\ V_D(0^+) = (1 - e^{[-\alpha d - \beta d^2]}) V(0^-) \\ V_N(0^+) = 0 \end{cases}, \tag{4}$$

where $V(0^-)$ is the spheroid volume before irradiation.

Equations (2), with their initial conditions (4), define a linear time-invariant dynamical system and (3) is the corresponding linear output equation. A block scheme of the basic model is represented in figure 1.

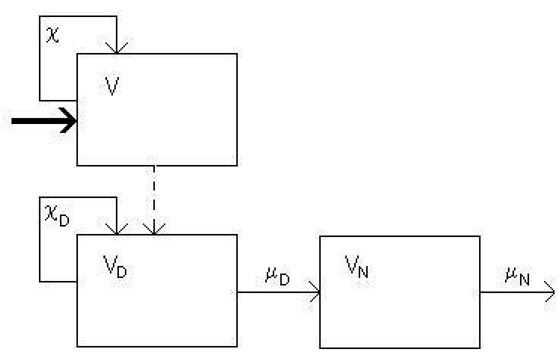

Figure 1: Block scheme of the basic model. The bold arrow entering the compartment of the viable cells represents the initial action of radiation dose d at 0^- and the vertical dashed arrow, between the compartments of viable cells and lethally damaged cells, represents the instantaneous redistribution of the cells at 0^+ between the two compartments, in agreement with the LQ model.

2.3 The model with subcompartments

Through the comparison with experimental data of the irradiated spheroid growth obtained from different tumour cell lines [10], it appears that the the basic model was not be able to fit some experimental trends of the irradiated spheroid growth. In fact, in some experimental data, the regression of the spheroid radius is not immediate after irradiation, but it is delayed [10], [2]. So in order to obtain a different dynamic, in which the peak of the exit flow from the compartments of lethally damaged cells and dead cells is delayed with respect to the irradiation time, it is convenient to divide the compartments of lethally damaged and dead cells into m subcompartments [2]. In the following we will restrict ourselves to consider the case of three subcompartments for both the compartments cited above.

The choice to modify the basic model introducing compartmental dynamic for the death of the lethally damaged cells and for the degradation of the dead cells, and the further choice to consider just three subcompartments, are justified by the analysis of the experimental data of different cell lines, as shown in [2], [4]. In fact with three subcompartments it has been obtained a significantly better fit than the previous model with only one compartment, particularly in the initial part of the treated response for high doses. Attempts with a larger number of subcompartments did not give appreciable improvements [2].

The equations of the model with subcompartments are:

$$\begin{cases} \dot{V}(t) = \chi V(t) \\ \dot{V}_{D_1}(t) = (\chi_D - \mu_D) V_{D_1}(t) \\ \dot{V}_{D_2}(t) = (\chi_D - \mu_D) V_{D_2}(t) + \mu_D V_{D_1}(t) \\ \dot{V}_{D_3}(t) = (\chi_D - \mu_D) V_{D_3}(t) + \mu_D V_{D_2}(t) \\ \dot{V}_{N_1}(t) = \mu_D V_{D_3}(t) - \mu_N V_{N_1}(t) \\ \dot{V}_{N_2}(t) = \mu_N V_{N_1}(t) - \mu_N V_{N_2}(t) \\ \dot{V}_{N_3}(t) = \mu_N V_{N_2}(t) - \mu_N V_{N_3}(t) \end{cases} , \tag{5}$$

with the output equation

$$y(t) = V(t) + V_{D_1}(t) + V_{D_2}(t) + V_{D_3}(t) + V_{N_1}(t) + V_{N_2}(t) + V_{N_3}(t) , \tag{6}$$

and the initial conditions

$$\begin{cases} V(0^+) = e^{[-\alpha d - \beta d^2]} V(0^-) \\ V_{D_1}(0^+) = (1 - e^{[-\alpha d - \beta d^2]}) V(0^-) \\ V_{D_2}(0^+) = 0 \\ V_{D_3}(0^+) = 0 \\ V_{N_1}(0^+) = 0 \\ V_{N_2}(0^+) = 0 \\ V_{N_3}(0^+) = 0 \end{cases} . \tag{7}$$

The modifications to the basic model neither modify the model structure, which remains linear and stationary, nor increase the number of parameters. In fact the model with subcompartments has the same parameters of the previous one (χ, χ_D, μ_D, μ_N, α and β), with the same biological meaning. A block scheme of the model with subcompartments is represented in figure 2.

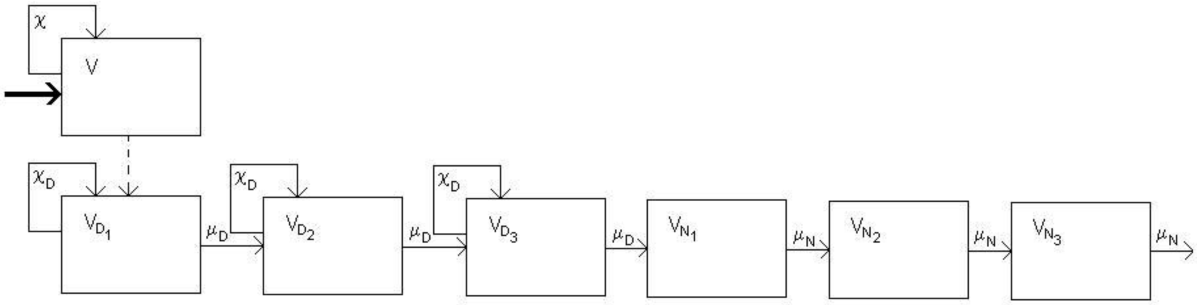

Figure 2: Block scheme of the model with subcompartments. The bold arrow entering the compartment of the viable cells represents the initial action of radiation dose d at 0^- and the vertical dashed arrow, between the compartment of viable cells and the first subcompartment of the lethally damaged cells, represents the instantaneous redistribution of the cells at 0^+ between the two compartments, in agreement with the LQ model.

3 Parametric identifiability

3.1 Identifiability of the basic model

A problem which constantly can be found in the mathematical modelling of biological systems (and more generally in all applicative fields of mathematical modelling) is that some model parameters are unknown. This problem takes on particular significance when the unknown parameters represent biological attributes which are not directly measurable, but are nevertheless of considerable scientific interest [11]. In this case we may wonder whether these parameters can be indirectly estimated by observing the system response to given inputs.

The identifiability refers to the possibility that the information obtainable from an experiment or from a set of experiments is sufficient to give a unique solution to the parameter values, in the absence of measurement noise. Therefore the main problem is to verify, before trying to estimate the unknown parameters, if the model structure allows this or, differently, if it gives no sense to it. The question of whether or not the unknown parameters of a dynamical system can be determined uniquely from an input-output relation constitutes the parametric identifiability problem.

Consider a general finite dimensional time-variant dynamical system depending on a parameter vector θ belonging to an admissible set $\Theta \subset R^\nu$:

$$\begin{cases} \dot{x}(t;\theta) = f(x(t;\theta), u(t), t, \theta), \qquad x(0^+;\theta) = h(x_{0^-}, \theta) \\ y(t;\theta) = g(x_{0^-}, u(t), t, \theta) \end{cases}$$

where the state vector $x(t;\theta) \in R^n$, the input $u(t) \in U \subset R^p$ and the output $y(t;\theta) \in R^q$. Denoting the couple (x_{0^-}, u) as an experiment, we can define an experiment set $E = \{(x_{0^-}, u)/x_{0^-} \in L \subset R^n; u(\cdot) \in \mathcal{U}([0,T])\}$, where $\mathcal{U}$ is a suitable class of functions with $u(t) \in U \subset R^p, t \in [0,T]$. So let us recall the following definitions:

Definition 1. The couple of parameter vector (θ, ϕ), with $\theta \in \Theta$ and $\phi \in \Theta$, is called indistinguishable with respect to the experiment set E if:

$$g(x_{0^-}, u(t), t, \theta) = g(x_{0^-}, u(t), t, \phi)\,, \quad \forall t \in [0, T] \ \mathit{and}\ \forall (x_{0^-}, u) \in E$$

Otherwise it is called distinguishable.

Definition 2. A parameter $\theta \in \Theta$ is locally identifiable if $\exists \varepsilon > 0$ such that the couple (θ, ϕ) is distinguishable for every $\phi \in S(\theta, \varepsilon) \cap \Theta, \phi \neq \theta$.

Definition 3. A parameter $\theta \in \Theta$ is globally identifiable if the couple (θ, ϕ) is distinguishable for every $\phi \in \Theta, \phi \neq \theta$.

There are different methods for the study of the identifiability of dynamical systems. For the models presented above it has been used the similarity transformation method [11], that can be only used for linear dynamical systems. In general, some of the matrix elements of a linear stationary dynamical system are not known. Therefore the similarity transformation method allows to determine the identifiability properties of system parameters when they correspond to the elements of the model matrices or when there is a univocal relationship between them. It is easy to understand, looking at the structure of the matrices given below, that a univocal relationship exists between the parameters $(\chi, \chi_D, \mu_D, \mu_N)$ and the elements of the system matrices whereas it does not happen for the radiological parameters (α, β). Considering the parameter δ, given by (1) and depending on the radiological parameters (α, β), even if it was identifiable, the parameters α and β would not be univocally determined from its value. It will be shown that α and β can be univocally identified by exploiting model responses to at least two different radiation doses.

Let us study, at first, the identifiability of the parameter vector θ of the basic model,

$$\theta = \begin{bmatrix} \chi \\ \chi_D \\ \mu_D \\ \mu_N \\ \delta \end{bmatrix}, \tag{8}$$

ranging in the admissible set $\Theta \subset R^5$, with

$$\Theta = \{\theta \in R^5 \mid \chi, \chi_D, \mu_D, \mu_N > 0, \quad \mu_D > \chi_D \ \text{ and } \ 0 < \delta < 1\}\,. \tag{9}$$

Denoting by

$$x(t) = \begin{bmatrix} V(t) \\ V_D(t) \\ V_N(t) \end{bmatrix} \tag{10}$$

the state vector of the basic model and by

$$A(\theta) = \begin{bmatrix} \chi & 0 & 0 \\ 0 & (\chi_D - \mu_D) & 0 \\ 0 & \mu_D & -\mu_N \end{bmatrix}, \quad c^T(\theta) = \begin{bmatrix} 1 & 1 & 1 \end{bmatrix},$$

$$b(\theta) = \begin{bmatrix} \delta \\ (1-\delta) \\ 0 \end{bmatrix}, \tag{11}$$

respectively the model dynamical matrix, the state-output matrix and the fraction of the initial state vector independent of the spheroid initial volume, the model can be rewritten as:

$$\begin{cases} \dot{x}(t;\theta) = A(\theta)x(t;\theta)\,, & x(0^+;\theta) = b(\theta)V(0^-) \\ y(t;\theta) = c^T(\theta)x(t;\theta) \end{cases} . \tag{12}$$

It is useful to observe, at this point, that the output $y(t;\theta)$ obtained by the model (12), in which no input acts, is the same output obtainable by the following model

$$\begin{cases} \dot{\bar{x}}(t;\theta) = A(\theta)\bar{x}(t;\theta) + b(\theta)u(t), & \bar{x}(0^-) = 0 \\ \bar{y}(t;\theta) = c^T(\theta)\bar{x}(t;\theta) \end{cases} , \tag{13}$$

with

$$u(t) = u_0(t)V(0^-)$$

where $u_0(t)$ is a Dirac unit pulse function. In fact:

$$y(t;\theta) = \bar{y}(t;\theta) = c^T(\theta)e^{A(\theta)t}b(\theta)V(0^-)\,. \tag{14}$$

Therefore, it is easy to understand from relation (14) that the identifiability problem of θ for the model (12) is the same one for the model (13). With reference to model (13), let us recall now the following definition:

Definition 4. The pair $(c^T(\theta), A(\theta))$ is observable if

$$\det \mathcal{O} = \det \begin{bmatrix} c^T(\theta) \\ c^T(\theta)A(\theta) \\ c^T(\theta)A^2(\theta) \end{bmatrix} \neq 0\,.$$

The pair $(A(\theta), b(\theta))$ is controllable if

$$\det \mathcal{C} = \det \begin{bmatrix} b(\theta) & A(\theta)b(\theta) & A^2(\theta)b(\theta) \end{bmatrix} \neq 0\,.$$

The triple $(A(\theta), b(\theta), c^T(\theta))$ is controllable and observable if the pair $(A(\theta), b(\theta))$ is controllable and the pair $(c^T(\theta), A(\theta))$ is observable.

The same definition can be applied to model (12). In particular we can talk about controllability of the couple $(A(\theta), b(\theta))$, since the role of the matrix $b(\theta)$ in the model (12) is equivalent to the one in the model (13).

The similarity transformation method is based on the following result:

Theorem 1. Let the triples $(A(\theta), b(\theta), c^T(\theta))$ and $(A(\phi), b(\phi), c^T(\phi))$ be observable and controllable. Then

$$c^T(\theta)e^{A(\theta)t}b(\theta) = c^T(\phi)e^{A(\phi)t}b(\phi)\,, \quad t \in [0, T] \tag{15}$$

if and only if a nonsingular matrix P exists such that

$$\begin{cases} PA(\theta)P^{-1} = A(\phi) \\ c^T(\theta)P^{-1} = c^T(\phi) \\ Pb(\theta) = b(\phi) \end{cases} . \tag{16}$$

Proof. It is immediate to see that (16) implies (15) by taking into account the power expansion of the exponential. The inverse implication, that requires the controllability and observability properties, was proved by Kalman [12], [13].

□

From theorem 1 it is easy to understand that given an indistinguishable couple $(\theta, \phi) \in \Theta$ for the system (12), if it exists, the corresponding system matrices, $(A(\theta), b(\theta), c^T(\theta))$ and $(A(\phi), b(\phi), c^T(\phi))$, have the same structure and are linked by the algebraic relations (16). It is easy also to see that if (16) have a unique solution (θ, I) then indistinguishable couples do not exist. So the following obvious lemmas follow:

Lemma 1. Let the triples $(A(\theta), b(\theta), c^T(\theta))$ and $(A(\phi), b(\phi), c^T(\phi))$ be observable and controllable. Then the system (12) is globally identifiable in Θ if and only if the equations (16), for all fixed vector $\theta \in \Theta$, have the unique solution $(\phi, P) = (\theta, I)$.

Lemma 2. Let the triples $(A(\theta), b(\theta), c^T(\theta))$ and $(A(\phi), b(\phi), c^T(\phi))$ be observable and controllable. Then the system (12) is locally identifiable in $S(\theta, \varepsilon) \cap \Theta$, for all θ in Θ and for $\varepsilon > 0$ sufficiently small, if and only if the equations (16) have isolated solutions in Θ for the unknown couples (ϕ, P), in addition to the trivial one (θ, I).

At this point, in order to study the identifiability property for the basic model (12) by the similarity transformation method it is necessary to verify, at first, the observability and the controllability properties for $\theta \in \Theta$. The observability matrix for the basic model is:

$$\mathcal{O}(\theta) = \begin{bmatrix} c^T(\theta) \\ c^T(\theta)A(\theta) \\ c^T(\theta)A^2(\theta) \end{bmatrix} = \begin{bmatrix} 1 & 1 & 1 \\ \chi & \chi_D & -\mu_N \\ \chi^2 & \chi_D(\chi_D - \mu_D) - \mu_D\mu_N & -\mu_N^2 \end{bmatrix} .$$

The determinant of $\mathcal{O}(\theta)$ is:

$$\det\{\mathcal{O}(\theta)\} = -(\chi + \mu_N)(\chi_D + \mu_N)(\chi + \mu_D - \chi_D) \tag{17}$$

and it is negative in Θ because all the factors are positive. Hence the couple $(c^T(\theta), A(\theta))$ is observable in Θ.

The controllability matrix for the basic model is:

$$\mathcal{C}(\theta) = \left[\begin{array}{ccc} b(\theta) & A(\theta)b(\theta) & A^2(\theta)b(\theta) \end{array}\right] =$$

$$= \left[\begin{array}{ccc} \delta & \chi\delta & \chi^2\delta \\ (1-\delta) & (\chi_D - \mu_D)(1-\delta) & (\chi_D - \mu_D)^2(1-\delta) \\ 0 & \mu_D(1-\delta) & \mu_D(\chi_D - \mu_D - \mu_N)(1-\delta) \end{array}\right].$$

The determinant of $\mathcal{C}(\theta)$ is:

$$\det\{\mathcal{C}(\theta)\} = \delta(1-\delta)^2\mu_D(\chi + \mu_N)(\chi + \mu_D - \chi_D) \tag{18}$$

and it is positive in Θ because all the factors are positive. Hence the couple $(A(\theta), b(\theta))$ is controllable in Θ.

Now we can prove the following result.

Theorem 2. The basic model (12) is locally (but not globally) identifiable with respect to the unknown parameter vector θ given by (8) and ranging in the set Θ defined by (9). In fact $\forall \theta \in \Theta$ there is a different parameter vector $\phi \in \Theta$ that gives the same output, $y(t;\theta) = y(t;\phi)$. These two points are isolated into Θ, so in a neighbourhood of θ, $S(\theta,\varepsilon) \cap \Theta$, θ is identifiable.

Proof. Given $\theta \in \Theta$, let us consider the (1×5) vector

$$\phi = \left[\begin{array}{c} \chi^* \\ \chi_D^* \\ \mu_D^* \\ \mu_N^* \\ \delta^* \end{array}\right] \in \Theta$$

and the (3×3) matrix

$$P = \left[\begin{array}{ccc} p_{11} & p_{12} & p_{13} \\ p_{21} & p_{22} & p_{23} \\ p_{31} & p_{32} & p_{33} \end{array}\right].$$

Rewriting the first equation of (16) as

$$PA(\theta) = A(\phi)P\,,$$

it is easy to obtain the following system of equations:

$$\begin{aligned} &\left[\begin{array}{ccc} p_{11}\chi & p_{12}(\chi_D - \mu_D) + p_{13}\mu_D & -p_{13}\mu_N \\ p_{21}\chi & p_{22}(\chi_D - \mu_D) + p_{23}\mu_D & -p_{23}\mu_N \\ p_{31}\chi & p_{32}(\chi_D - \mu_D) + p_{33}\mu_D & -p_{33}\mu_N \end{array}\right] = \\ &= \left[\begin{array}{ccc} p_{11}\chi^* & p_{12}\chi^* & p_{13}\chi^* \\ p_{21}(\chi_D^* - \mu_D^*) & p_{22}(\chi_D^* - \mu_D^*) & p_{23}(\chi_D^* - \mu_D^*) \\ p_{21}\mu_D^* - p_{31}\mu_N^* & p_{22}\mu_D^* - p_{32}\mu_N^* & p_{23}\mu_D^* - p_{33}\mu_N^* \end{array}\right]. \end{aligned} \tag{19}$$

Furthermore, rewriting the second equation of (16) as

$$c^T(\theta) = c^T(\phi)P$$

and using also the third equation, it is easy to obtain, respectively, the following relations:

$$\begin{cases} p_{11} + p_{21} + p_{31} = 1 \\ p_{12} + p_{22} + p_{32} = 1 \\ p_{13} + p_{23} + p_{33} = 1 \end{cases} \tag{20}$$

$$\begin{cases} p_{11}\delta + p_{12}(1-\delta) = \delta^* \\ p_{21}\delta + p_{22}(1-\delta) = (1-\delta^*) \\ p_{31}\delta + p_{32}(1-\delta) = 0 \end{cases} \tag{21}$$

From the equality of the elements of position $(1,3)$ and of the elements of position $(2,1)$ of the system (19), we obtain

$$p_{13}(\chi^* + \mu_N) = 0, \quad p_{21}(\chi + \mu_D^* - \chi_D^*) = 0,$$

and keeping in mind the parameter constraints of Θ, it results that $p_{13} = p_{21} = 0$. Using these first results and equalling the elements of position $(1,2)$ and then the elements of position $(3,1)$ of (19), it is easy to show that also $p_{12} = p_{31} = 0$. From the first equation of (20) it results that $p_{11} = 1$ while from the (21), using the value of the five elements of P obtained, it is immediate to obtain, respectively, that $\delta^* = \delta$, $p_{22} = 1$ and $p_{32} = 0$. Hence, until this point, the following results are obtained:

$$P = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & p_{23} \\ 0 & 0 & p_{33} \end{bmatrix}, \quad \text{with} \quad \begin{cases} \chi^* = \chi \\ \delta^* = \delta \end{cases}$$

and the relations that remain are:

$$\begin{bmatrix} (\chi_D - \mu_D) + p_{23}\mu_D & -p_{23}\mu_N \\ p_{33}\mu_D & -p_{33}\mu_N \end{bmatrix} = \begin{bmatrix} (\chi_D^* - \mu_D^*) & p_{23}(\chi_D^* - \mu_D^*) \\ \mu_D^* & p_{23}\mu_D^* - p_{33}\mu_N^* \end{bmatrix} \tag{22}$$

$$p_{23} + p_{33} = 1 \tag{23}$$

By adding and then equalling the elements $(1,1)$ and $(2,1)$ of (22) it results that

$$\chi_D^* = \chi_D\,.$$

By summing and then setting equal the elements $(1,2)$ and $(2,2)$ of (22) it is easy to obtain the following expression for μ_N^*

$$\mu_N^* = \frac{p_{23}\chi_D + \mu_N}{p_{33}},$$

and from the elements $(2,1)$ of (22) it results that

$$\mu_D^* = p_{33}\mu_D\,.$$

From the equality of the elements $(2,2)$ of (22) and replacing the expressions obtained for μ_D^* and μ_N^*, it results that

$$(1-p_{33})\mu_N - p_{23}p_{33}\mu_D + p_{23}\chi_D = 0$$

from which, recalling (23), it results that two different solutions for the couple (p_{23}, p_{33}) exist:

$$p_{23} = 0, p_{33} = 1 \quad or \quad p_{23} = \frac{\mu_D - \chi_D - \mu_N}{\mu_D}, p_{33} = \frac{\chi_D + \mu_D}{\mu_D}.$$

In conclusion we have that system (16) admits the two different solutions:

$$\begin{cases} (\theta, I) \\ and \\ \left(\begin{bmatrix} \chi \\ \chi_D \\ \mu_N + \chi_D \\ \mu_D - \chi_D \\ \delta \end{bmatrix}, \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{\mu_D - \chi_D - \mu_N}{\mu_D} \\ 0 & 0 & \frac{\chi_D + \mu_D}{\mu_D} \end{bmatrix} \right) \end{cases}, \tag{24}$$

which are both admissible, since the vectors belong to Θ and the matrices are non singular. So, for lemma 2, we can say that the basic model is locally identifiable with respect to the five considered parameters.

$\square$

This result can be easily confirmed looking at the explicit expression of the output $y(t;\theta)$. In fact, for the basic model, it is easy to integrate the dynamical equations obtaining the following expression:

$$y(t;\theta) = [\delta e^{\chi t} - \frac{(1-\delta)\mu_D}{\chi_D - \mu_D + \mu_N} e^{-\mu_N t} + \frac{\chi_D + \mu_N}{\chi_D - \mu_D + \mu_N} e^{(\chi_D - \mu_D)t}] V(0^-)$$

from which it is immediate to verify that $y(t;\theta) = y(t;\phi)$, if the vector ϕ is such that

$$\begin{cases} \chi^* = \chi \\ \chi_D^* = \chi_D \\ \mu_D^* = \mu_N + \chi_D \\ \mu_N^* = \mu_D - \chi_D \\ \delta^* = \delta \end{cases}$$

In order to study the identifiability of the radiological parameters α and β it is necessary to consider two different doses d_1 and d_2 and the corresponding parameters δ_1 and δ_2 that depend univocally from the couple (α, β):

$$\begin{cases} \delta_1 = e^{[-\alpha d_1 - \beta d_1^2]} \\ \delta_2 = e^{[-\alpha d_2 - \beta d_2^2]} \end{cases} \tag{25}$$

It is easy to verify that the relation between (δ_1, δ_2) and (α, β) is one to one if $d_1 \neq d_2$. In fact, from (25) it results

$$\begin{bmatrix} -d_1 & -d_1^2 \\ -d_2 & -d_2^2 \end{bmatrix} \begin{bmatrix} \alpha \\ \beta \end{bmatrix} = \begin{bmatrix} \ln(\delta_1) \\ \ln(\delta_2) \end{bmatrix}$$

which admits a unique solution for the couple (α, β) provided that $d_1 \neq d_2$. Therefore, let us consider two different initial states $x^{(1)}(0^+; \theta)$ and $x^{(2)}(0^+; \theta)$ related to two different doses and let us observe parallely the two corresponding system responses. Let us define a new parameter vector

$$\theta = \begin{bmatrix} \chi \\ \chi_D \\ \mu_D \\ \mu_N \\ \delta_1 \\ \delta_2 \end{bmatrix} \tag{26}$$

ranging in the admissible set $\Theta \subset R^6$, with

$$\Theta = \{\theta \in R^6 \mid \chi, \chi_D, \mu_D, \mu_N > 0, \quad \mu_D > \chi_D, \quad 0 < \delta_1 < 1 \text{ and } 0 < \delta_2 < 1\}. \tag{27}$$

Let us define by $x_T(t) \in R^6$ the total state vector

$$x_T(t) = \begin{bmatrix} x^{(1)}(t) \\ x^{(2)}(t) \end{bmatrix}, \tag{28}$$

that is the union of two state vectors of the type (10), $x^{(1)}(t)$ and $x^{(2)}(t)$, related to the two different initial states, and the block matrices

$$\begin{gathered} A_T(\theta) = \begin{bmatrix} A(\theta) & 0 \\ 0 & A(\theta) \end{bmatrix}, \quad C_T(\theta) = \begin{bmatrix} c^T(\theta) & 0 \\ 0 & c^T(\theta) \end{bmatrix}, \\ B_T(\theta) = \begin{bmatrix} b^{(1)}(\theta) & 0 \\ 0 & b^{(2)}(\theta) \end{bmatrix}, \end{gathered} \tag{29}$$

where $A(\theta)$ and $c^T(\theta)$ are the same matrix defined in (11), and $b^{(1)}(\theta)$, $b^{(2)}(\theta)$ are such that

$$b^{(1)}(\theta) = \begin{bmatrix} \delta_1 \\ (1-\delta_1) \\ 0 \end{bmatrix}, \quad b^{(2)}(\theta) = \begin{bmatrix} \delta_2 \\ (1-\delta_2) \\ 0 \end{bmatrix}. \tag{30}$$

Obviously, $A_T(\theta)$, $C_T(\theta)$ and $B_T(\theta)$ are, respectively, (6×6), (2×6) and (6×2) matrix and the basic extended model can be written as:

$$\begin{cases} \dot{x}_T(t;\theta) = A_T(\theta) x_T(t;\theta), \qquad x_T(0^+;\theta) = B_T(\theta) \begin{pmatrix} V(0^-) \\ V(0^-) \end{pmatrix} \\ y_T(t;\theta) = C_T(\theta) x_T(t;\theta) \end{cases}, \tag{31}$$

where $y_T(t;\theta) \in R^2$ is the union of the two outputs related to the two different initial states.

The observability matrix of the couple $(C_T(\theta), A_T(\theta))$ is

$$\mathcal{O}_T(\theta) = \begin{bmatrix} C_T(\theta) \\ C_T(\theta)A_T(\theta) \\ \vdots \\ C_T(\theta)A_T^5(\theta) \end{bmatrix} = \begin{bmatrix} c^T(\theta) & 0 \\ 0 & c^T(\theta) \\ c^T(\theta)A(\theta) & 0 \\ 0 & c^T(\theta)A(\theta) \\ \vdots & \vdots \\ c^T(\theta)A^5(\theta) & 0 \\ 0 & c^T(\theta)A^5(\theta) \end{bmatrix}.$$

that is a (12×6) matrix from which it is possible to extract the (6×6) minor

$$M = \begin{bmatrix} c^T(\theta) & 0 \\ c^T(\theta)A(\theta) & 0 \\ c^T(\theta)A^2(\theta) & 0 \\ 0 & c^T(\theta) \\ 0 & c^T(\theta)A(\theta) \\ 0 & c^T(\theta)A^2(\theta) \end{bmatrix}.$$

that is non singular in Θ because it is

$$\det\{M\} = [\det\{\mathcal{O}(\theta)\}]^2$$

and $\det\{\mathcal{O}(\theta)\}$ is different from zero, as shown in (17) and commented above.

Similarly the controllability matrix of the couple $(A_T(\theta), B_T(\theta))$ is

$$\mathcal{C}_T(\theta) = \begin{bmatrix} B_T(\theta) & A_T(\theta)B_T(\theta) & ... & A_T^5(\theta)B_T(\theta) \end{bmatrix} =$$

$$= \begin{bmatrix} b^{(1)}(\theta) & 0 & A(\theta)b^{(1)}(\theta) & 0 & ... & A^5(\theta)b^{(1)}(\theta) & 0 \\ 0 & b^{(2)}(\theta) & 0 & A(\theta)b^{(2)}(\theta) & ... & 0 & A^5(\theta)b^{(2)}(\theta) \end{bmatrix},$$

which is a (6×12) matrix from which it is possible to extract the (6×6) minor

$$N = \begin{bmatrix} b^{(1)}(\theta) & A(\theta)b^{(1)}(\theta) & A^2(\theta)b^{(1)}(\theta) & 0 & 0 & 0 \\ 0 & 0 & 0 & b^{(2)}(\theta) & A(\theta)b^{(2)}(\theta) & A^2(\theta)b^{(2)}(\theta) \end{bmatrix},$$

which is non singular. In fact its determinant is

$$\det\{N\} = \det\{\mathcal{C}^{(1)}(\theta)\} \cdot \det\{\mathcal{C}^{(2)}(\theta)\}$$

where $\det\{\mathcal{C}^{(1)}(\theta)\}$ and $\det\{\mathcal{C}^{(2)}(\theta)\}$ are the determinants of the controllability matrices related to the doses d_1 and d_2, respectively, and are different from zero, as already shown in (18). So (31) is observable and controllable.

Now we can prove the following result.

Theorem 3. The basic model (12) is locally (but not globally) identifiable with respect to the unknown parameter vector θ given by (26) and ranging in the set Θ defined by (27), exploiting the outputs of the model obtained from two different radiation doses.

Proof. Given $\theta \in \Theta$, let us consider the (1×6) vector

$$\phi = \begin{bmatrix} \chi^* \\ \chi_D^* \\ \mu_D^* \\ \mu_N^* \\ \delta_1^* \\ \delta_2^* \end{bmatrix} \in \Theta$$

and the (6×6) matrix

$$P = \begin{bmatrix} p_{11} & p_{12} & \cdots & p_{16} \\ p_{21} & p_{22} & \cdots & p_{26} \\ p_{31} & p_{32} & \cdots & p_{36} \\ p_{41} & p_{42} & \cdots & p_{46} \\ p_{51} & p_{52} & \cdots & p_{56} \\ p_{61} & p_{62} & \cdots & p_{66} \end{bmatrix}.$$

Dividing the P matrix into four (3×3) blocks

$$P = \begin{bmatrix} P_{11} & P_{12} \\ P_{21} & P_{22} \end{bmatrix} \tag{32}$$

it is easy to show that from the matrix equations (16), developing the block products, the following subsystems are obtained:

$$\begin{array}{ll} (a) \begin{cases} P_{11}A(\theta) = A(\phi)P_{11} \\ c^T(\theta) = c^T(\phi)P_{11} \\ P_{11}b^{(1)}(\theta) = b^{(1)}(\phi) \end{cases} & (b) \begin{cases} P_{12}A(\theta) = A(\phi)P_{12} \\ 0 = c^T(\phi)P_{12} \\ P_{12}b^{(2)}(\theta) = 0 \end{cases} \\ \\ (c) \begin{cases} P_{21}A(\theta) = A(\phi)P_{21} \\ 0 = c^T(\phi)P_{21} \\ P_{21}b^{(1)}(\theta) = 0 \end{cases} & (d) \begin{cases} P_{22}A(\theta) = A(\phi)P_{22} \\ c^T(\theta) = c^T(\phi)P_{22} \\ P_{22}b^{(2)}(\theta) = b^{(2)}(\phi) \end{cases} \end{array} \tag{33}$$

The subsystems (a) and (d) of (33) are similar to the one studied in the proof of theorem 2. So both the subsystems have two solutions of the type (24). So from (a) and (d) it results that

$$\begin{cases} \chi^* = \chi \\ \chi_D^* = \chi_D \\ \mu_D^* = \mu_D \\ \mu_N^* = \mu_N \\ \delta_1^* = \delta_1 \\ \delta_2^* = \delta_2 \\ P_{11} = P_{22} = I \end{cases} \quad or \quad \begin{cases} \chi^* = \chi \\ \chi_D^* = \chi_D \\ \mu_D^* = \mu_N + \chi_D \\ \mu_N^* = \mu_D - \chi_D \\ \delta_1^* = \delta_1 \\ \delta_2^* = \delta_2 \\ P_{11} = P_{22} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & \frac{\mu_D - \chi_D - \mu_N}{\mu_D} \\ 0 & 0 & \frac{\chi_D + \mu_D}{\mu_D} \end{bmatrix} \end{cases}.$$

Moreover, since the two subsystems (b) and (c) of (33) are similar, it is sufficient to study one of them, for instance the problem (b). From the first solution obtained from (a) and (d) of (33), the system (b) can be rewritten as

$$\begin{bmatrix} p_{44}\chi & p_{45}(\chi_D-\mu_D)+p_{46}\mu_D & -p_{46}\mu_N \\ p_{54}\chi & p_{55}(\chi_D-\mu_D)+p_{56}\mu_D & -p_{56}\mu_N \\ p_{64}\chi & p_{65}(\chi_D-\mu_D)+p_{66}\mu_D & -p_{66}\mu_N \end{bmatrix} =$$

$$= \begin{bmatrix} p_{44}\chi & p_{45}\chi & p_{46}\chi \\ p_{54}(\chi_D-\mu_D) & p_{55}(\chi_D-\mu_D) & p_{56}(\chi_D-\mu_D) \\ p_{54}\mu_D-p_{64}\mu_N & p_{55}\mu_D-p_{65}\mu_N & p_{56}\mu_D-p_{66}\mu_N \end{bmatrix}, \tag{34}$$

with

$$\begin{cases} p_{44}+p_{54}+p_{64}=0 \\ p_{45}+p_{55}+p_{65}=0 \\ p_{46}+p_{56}+p_{66}=0 \end{cases}, \quad \begin{cases} p_{44}\delta+p_{45}(1-\delta)=0 \\ p_{54}\delta+p_{55}(1-\delta)=0 \\ p_{64}\delta+p_{65}(1-\delta)=0 \end{cases}. \tag{35}$$

Equalling the elements $(1,3)$ and then the elements $(2,1)$ of (34), it results that $p_{46}=p_{54}=0$. Applying the same procedure to the elements $(1,2)$ and then $(3,1)$ of (34), it follows that $p_{45}=p_{64}=0$. Through (35) it is easy to verify also that $p_{44}=p_{55}=p_{56}=p_{65}=p_{66}=0$.

Similarly, from the second solution obtained from (a) and (d) of (33), the system (c), taking into account that

$$A(\phi)=\begin{bmatrix} \chi & 0 & 0 \\ 0 & -\mu_N & 0 \\ 0 & (\mu_N+\chi_D) & (\chi_D-\mu_D) \end{bmatrix},$$

can be rewritten as

$$\begin{bmatrix} p_{44}\chi & p_{45}(\chi_D-\mu_D)+p_{46}\mu_D & -p_{46}\mu_N \\ p_{54}\chi & p_{55}(\chi_D-\mu_D)+p_{56}\mu_D & -p_{56}\mu_N \\ p_{64}\chi & p_{65}(\chi_D-\mu_D)+p_{66}\mu_D & -p_{66}\mu_N \end{bmatrix} =$$

$$= \begin{bmatrix} p_{44}\chi & p_{45}\chi & p_{46}\chi \\ -p_{54}\mu_N & -p_{55}\mu_N & -p_{56}\mu_N \\ p_{54}(\mu_N+\chi_D)+p_{64}(\chi_D-\mu_D) & p_{55}(\mu_N+\chi_D)+p_{65}(\chi_D-\mu_D) & p_{56}(\mu_N+\chi_D)+p_{66}(\chi_D-\mu_D) \end{bmatrix},$$

with

$$\begin{cases} p_{44}+p_{54}+p_{64}=0 \\ p_{45}+p_{55}+p_{65}=0 \\ p_{46}+p_{56}+p_{66}=0 \end{cases}, \quad \begin{cases} p_{44}\delta+p_{45}(1-\delta)=0 \\ p_{54}\delta+p_{55}(1-\delta)=0 \\ p_{64}\delta+p_{65}(1-\delta)=0 \end{cases}.$$

Proceeding as for (34) and (35), it is easy to obtain that the (3×3) matrix $P_{12}=0$. So, for both the solutions of (a) and (d) of (33), the solutions of the subsystems (b) and (c) of (33) are the matrices $P_{12}=P_{21}=0$.

In conclusion we have that system (16) admits the two different solutions:

$$\begin{cases} (\theta, I) \\ and \\ \left(\begin{bmatrix} \chi \\ \chi_D \\ \mu_N + \chi_D \\ \mu_D - \chi_D \\ \delta_1 \delta_2 \end{bmatrix}, \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & \frac{\mu_D - \chi_D - \mu_N}{\mu_D} & 0 & 0 & 0 \\ 0 & 0 & \frac{\chi_D + \mu_D}{\mu_D} & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & \frac{\mu_D - \chi_D - \mu_N}{\mu_D} \\ 0 & 0 & 0 & 0 & 0 & \frac{\chi_D + \mu_D}{\mu_D} \end{bmatrix} \right) \end{cases}.$$

which are both admissible, since the the vectors belong to Θ and the matrices are non singular. So, for lemma 2, we can say that model (12) is locally identifiable by exploiting the model response $y_T(t; \theta)$ to at least two different doses d_1 and d_2.

□

3.2 Identifiability of the model with subcompartments

In this section it will be proved the global identifiability of the model (5) - (7). Let us study at first, as for the previous case, the identifiability of the parameter vector θ,

$$\theta = \begin{bmatrix} \chi \\ \chi_D \\ \mu_D \\ \mu_N \\ \delta \end{bmatrix}, \tag{36}$$

ranging in the admissible set $\Theta \subset R^5$, with

$$\Theta = \{\theta \in R^5 \mid \chi, \chi_D, \mu_D, \mu_N > 0, \quad \mu_D > \chi_D \text{ and } 0 < \delta < 1\}. \tag{37}$$

Denoting by

$$x(t) = \begin{bmatrix} V(t) \\ V_{D_1}(t) \\ V_{D_2}(t) \\ V_{D_3}(t) \\ V_{N_1}(t) \\ V_{N_2}(t) \\ V_{N_3}(t) \end{bmatrix} \tag{38}$$

the state vector of the model with three subcompartments, and by

$$A(\theta) = \begin{bmatrix} \chi & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & (\chi_D - \mu_D) & 0 & 0 & 0 & 0 & 0 \\ 0 & \mu_D & (\chi_D - \mu_D) & 0 & 0 & 0 & 0 \\ 0 & 0 & \mu_D & (\chi_D - \mu_D) & 0 & 0 & 0 \\ 0 & 0 & 0 & \mu_D & -\mu_N & 0 & 0 \\ 0 & 0 & 0 & 0 & \mu_N & -\mu_N & 0 \\ 0 & 0 & 0 & 0 & 0 & \mu_N & -\mu_N \end{bmatrix},$$

$$c^T(\theta) = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}, \tag{39}$$

$$b(\theta) = \begin{bmatrix} \delta \\ (1-\delta) \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix},$$

respectively the model dynamical matrix, the state-output matrix and the fraction of the initial state vector independent of the spheroid initial volume, the model (5) - (7) can be again rewritten in the compact form (12).

In order to prove the identifiability property of this model by the similarity transformation method it is necessary to verify the observability and the controllability properties of the system, for $\theta \in \Theta$. With reference to the observability matrix $\mathcal{O}(\theta)$ of the couple $(c^T(\theta), A(\theta))$, we obtain by symbolic computation (using MATLAB 7.6):

$$\begin{array}{l} \det\{\mathcal{O}(\theta)\} = -\mu_N^3\mu_D^3(\chi + \mu_D - \chi_D)^3(\chi + \mu_N)^3 \\ (\mu_N^3 + 3\chi_D\mu_N^2 - 3\mu_N\chi_D\mu_D + 3\mu_N\chi_D^2 + \chi_D^3 - 2\chi_D^2\mu_D + \chi_D\mu_D)^3 \end{array}, \tag{40}$$

and it is easy to verify that, for $\theta \in \Theta$, each factor of the above expression is positive. In particular the last factor can be written in the following form, by defining $X = \mu_D/\chi_D > 1$ and $Y = \mu_N/\chi_D > 0$:

$$\chi_D^9[X^2 - (2 + 3Y)X + (1 + Y)^3]^3,$$

and it is immediate to verify that the second-order polynomial in X in square brackets is always positive for $Y > 0$. Hence the couple $(c^T(\theta), A(\theta))$ is observable in Θ.

The determinant of the controllability matrix $\mathcal{C}(\theta)$ is similarly obtained by a symbolic computation:

$$\det\{\mathcal{C}(\theta)\} = \delta(1-\delta)^6\mu_D^{12}\mu_N^3(\chi + \mu_N)^3(\chi + \mu_D - \chi_D)^3 \tag{41}$$

and it is positive in Θ because all the factors are positive. Hence the couple $(A(\theta), b(\theta))$ is controllable in Θ.

Now we can prove the following result.

Theorem 4. The model (5) - (7) is globally identifiable with respect to the unknown parameter vector θ given by (36) and ranging in the set Θ defined by (37). In fact $\forall \theta \in \Theta$ it does not exist in Θ another parameter vector ϕ that gives the same output.

Proof. Given $\theta \in \Theta$, let us consider the (1×5) vector

$$\phi = \begin{bmatrix} \chi^* \\ \chi_D^* \\ \mu_D^* \\ \mu_N^* \\ \delta^* \end{bmatrix} \in \Theta$$

and the (7×7) matrix

$$P = \begin{bmatrix} p_{11} & p_{12} & \cdots & p_{17} \\ p_{21} & p_{22} & \cdots & p_{27} \\ p_{31} & p_{32} & \cdots & p_{37} \\ p_{41} & p_{42} & \cdots & p_{47} \\ p_{51} & p_{52} & \cdots & p_{57} \\ p_{61} & p_{62} & \cdots & p_{67} \\ p_{71} & p_{72} & \cdots & p_{77} \end{bmatrix}.$$

From the first equation of (16) it is easy to obtain the following equation system:

$$\begin{bmatrix} \mathfrak{r}_1 \\ \mathfrak{r}_2 \\ \mathfrak{r}_3 \\ \mathfrak{r}_4 \\ \mathfrak{r}_5 \\ \mathfrak{r}_6 \\ \mathfrak{r}_7 \end{bmatrix} = \begin{bmatrix} \mathfrak{c}_1 & \mathfrak{c}_2 & \mathfrak{c}_3 & \mathfrak{c}_4 & \mathfrak{c}_5 & \mathfrak{c}_6 & \mathfrak{c}_7 \end{bmatrix}, \tag{42}$$

where $\mathfrak{r}_i$ is a (1×7) row vector

$$\mathfrak{r}_i = \begin{bmatrix} p_{i1}\chi \\ p_{i2}(\chi_D - \mu_D) + p_{i3}\mu_D \\ p_{i3}(\chi_D - \mu_D) + p_{i4}\mu_D \\ p_{i4}(\chi_D - \mu_D) + p_{i5}\mu_D \\ -p_{i5}\mu_N + p_{i6}\mu_N \\ -p_{i6}\mu_N + p_{i7}\mu_N \\ -p_{i7}\mu_N \end{bmatrix}^T, \quad i = 1, 2, ..., 7,$$

and $\mathfrak{c}_i$ is a (7×1) column vector

$$\mathfrak{c}_i = \begin{bmatrix} p_{1i}\chi^* \\ p_{2i}(\chi_D^* - \mu_D^*) \\ p_{2i}\mu_D^* + p_{3i}(\chi_D^* - \mu_D^*) \\ p_{3i}\mu_D^* + p_{4i}(\chi_D^* - \mu_D^*) \\ p_{4i}\mu_D^* - p_{5i}\mu_N^* \\ p_{5i}\mu_N^* - p_{6i}\mu_N^* \\ p_{6i}\mu_N^* - p_{7i}\mu_N^* \end{bmatrix}, \quad i = 1, 2, ..., 7.$$

Furthermore, using the second and the third equation of (16) it is easy to obtain, respectively, the following relations:

$$\begin{cases} p_{11} + p_{21} + ... + p_{71} = 1 \\ p_{12} + p_{22} + ... + p_{72} = 1 \\ \vdots \\ p_{17} + p_{27} + ... + p_{77} = 1 \end{cases} \tag{43}$$

$$\begin{cases} p_{11}\delta + p_{12}(1-\delta) = \delta^* \\ p_{21}\delta + p_{22}(1-\delta) = (1-\delta^*) \\ p_{31}\delta + p_{32}(1-\delta) = 0 \\ \vdots \\ p_{71}\delta + p_{72}(1-\delta) = 0 \end{cases} \tag{44}$$

From the equality of the elements of position $(1,7)$ and of the elements of position $(2,1)$ of the system (42) we have

$$p_{17}(\chi^* + \mu_N) = 0, \quad p_{21}(\chi + \mu_D^* - \chi_D^*) = 0,$$

and keeping in mind the parameter constraints of Θ, it results that $p_{17} = p_{21} = 0$. Using these first results and equalling the elements of the first row of both sides of (42) and then the elements of the first column of both sides of (42), it is easy to show that $p_{12} = p_{13} = p_{14} = p_{15} = p_{16} = p_{31} = p_{41} = p_{51} = p_{61} = p_{71} = 0$. From the first equation of (43) it results that $p_{11} = 1$; using this value and setting equal the $(1,1)$ element of (42) it results that $\chi^* = \chi$. So, from system (44) it is immediate to obtain that $\delta^* = \delta$, $p_{22} = 1$ and $p_{32} = p_{42} = p_{52} = p_{62} = p_{72} = 0$.

Now, equalling the elements under the principal subdiagonal of (42) it is easy to obtain that $p_{43} = p_{53} = p_{63} = p_{73} = 0$ from the equalities of the second column, $p_{54} = p_{64} = p_{74} = 0$ from the equalities of the third column, $p_{65} = p_{75} = 0$ and $p_{76} = 0$, respectively from the equalities of the fourth and fifth column. So, at this point, by summing the elements of the second column of the left-hand of (42) and of the right-hand of (42), and then setting them equal, it is easy to obtain that $\chi_D^* = \chi_D$. Repeating the same procedure for the fifth column of both sides of (42) we have

$$(p_{25} + p_{35} + p_{45})\chi_D = 0$$

from which, since $\chi_D > 0$

$$p_{25} + p_{35} + p_{45} = 0\,. \tag{45}$$

From (45) and from the fifth equation of (43) it follows that $p_{55} = 1$. Equalling the elements $(3,2)$, $(4,3)$ and $(5,4)$ of (42) it can be obtained respectively that

$$p_{33}\mu_D = \mu_D^*, \quad p_{44}\mu_D = p_{33}\mu_D^*, \quad \mu_D = p_{44}\mu_D^*$$

which imply that $\mu_D^* = \mu_D$ and $p_{33} = p_{44} = 1$. At this point, equalling the elements of the second, the third and the forth row of the both sides of (42), we obtain that $p_{23} = p_{24} = p_{25} = p_{26} = p_{27} = 0$, $p_{34} = p_{35} = p_{36} = p_{37} = 0$ and $p_{45} = p_{46} = p_{47} = 0$.

At this point we have obtained that

$$P = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & p_{56} & p_{57} \\ 0 & 0 & 1 & 0 & 0 & p_{66} & p_{67} \\ 0 & 0 & 0 & 0 & 0 & 0 & p_{77} \end{bmatrix}, \quad \text{with} \quad \begin{cases} \chi^* = \chi \\ \chi_D^* = \chi_D \\ \mu_D^* = \mu_D \\ \delta^* = \delta \end{cases}$$

and the available equations are:

$$\begin{bmatrix} -\mu_N + p_{56}\mu_N & -p_{56}\mu_N + p_{57}\mu_N & -p_{57}\mu_N \\ p_{66}\mu_N & -p_{66}\mu_N + p_{67}\mu_N & -p_{67}\mu_N \\ 0 & p_{77}\mu_N & -p_{77}\mu_N \end{bmatrix} =$$
$$= \begin{bmatrix} -\mu_N^* & -p_{56}\mu_N^* & -p_{57}\mu_N^* \\ \mu_N^* & p_{56}\mu_N^* - p_{66}\mu_N^* & p_{57}\mu_N^* - p_{67}\mu_N^* \\ 0 & p_{66}\mu_N^* & p_{67}\mu_N^* - p_{77}\mu_N^* \end{bmatrix} \tag{46}$$

and

$$\begin{cases} p_{56} + p_{66} = 1 \\ p_{57} + p_{67} + p_{77} = 1 \end{cases} . \tag{47}$$

By summing the elements of the third column of the left-hand of (46) and of the right-hand of (46), and then making them equal, it is easy to obtain that

$$\mu_N = p_{77}\mu_N^* \tag{48}$$

and then making the elements $(2, 1)$ and $(3, 2)$ equal we obtain

$$p_{66}\mu_N = \mu_N^*, \quad p_{77}\mu_N = p_{66}\mu_N^* \tag{49}$$

From (48) and (49) it follows that $p_{66} = p_{77} = 1$ and $\mu_N^* = \mu_N$. With these last results, from (46) and (47), it is immediate to verify that $p_{56} = p_{57} = p_{67} = 0$.

Therefore we have that system (16) admits only the trivial solution (θ, I). So, from lemma 1, we can say that the model (5) - (7) is globally identifiable with respect to the five considered parameters.

□

Theorem 4 does not establish the global identifiability of the model (5) - (7) with respect to the radiological parameters. In order to obtain the identifiability of the couple (α, β) we again have to consider two different doses and the corresponding parameters δ_1 and δ_2 that depend univocally from the couple (α, β), as done previously for the basic model. So, let us consider two different initial states $x^{(1)}(0^+; \theta)$ and $x^{(2)}(0^+; \theta)$ related to the two different doses and let us observe parallely the two corresponding system responses. Let us define a new parameter vector

$$\theta = \begin{bmatrix} \chi \\ \chi_D \\ \mu_D \\ \mu_N \\ \delta_1 \\ \delta_2 \end{bmatrix} \tag{50}$$

ranging in the admissible set $\Theta \subset R^6$, with

$$\Theta = \{\theta \in R^6 \mid \chi, \chi_D, \mu_D, \mu_N > 0, \quad \mu_D > \chi_D, \quad 0 < \delta_1 < 1 \ \text{ and } \ 0 < \delta_2 < 1\}\,. \tag{51}$$

Let us define by $x_T(t) \in R^{14}$ the total state vector

$$x_T(t) = \begin{bmatrix} x^{(1)}(t) \\ x^{(2)}(t) \end{bmatrix}, \tag{52}$$

that is the union of two state vectors of the type (38), $x^{(1)}(t)$ and $x^{(2)}(t)$, related to the two different initial states, and the block matrices

$$\begin{gathered} A_T(\theta) = \begin{bmatrix} A(\theta) & 0 \\ 0 & A(\theta) \end{bmatrix}, \quad C_T(\theta) = \begin{bmatrix} c^T(\theta) & 0 \\ 0 & c^T(\theta) \end{bmatrix}, \\ B_T(\theta) = \begin{bmatrix} b^{(1)}(\theta) & 0 \\ 0 & b^{(2)}(\theta) \end{bmatrix}, \end{gathered} \tag{53}$$

where $A(\theta)$ and $c^T(\theta)$ are the same matrix defined in (39), $b^{(1)}(\theta)$ and $b^{(2)}(\theta)$ are such that

$$b^{(1)}(\theta) = \begin{bmatrix} \delta_1 \\ (1-\delta_1) \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \quad b^{(2)}(\theta) = \begin{bmatrix} \delta_2 \\ (1-\delta_2) \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}. \tag{54}$$

Hence, the extended model with subcompartments can be written as in (31), with $A_T(\theta)$, $C_T(\theta)$ and $B_T(\theta)$ that are now, respectively, the (14×14), (2×14) and (14×2) matrices shown in (53).

The observability matrix of the couple $(C_T(\theta), A_T(\theta))$ is the (28×14) matrix

$$\mathcal{O}_T(\theta) = \begin{bmatrix} c^T(\theta) & 0 \\ 0 & c^T(\theta) \\ c^T(\theta)A(\theta) & 0 \\ 0 & c^T(\theta)A(\theta) \\ \vdots & \vdots \\ c^T(\theta)A^{13}(\theta) & 0 \\ 0 & c^T(\theta)A^{13}(\theta) \end{bmatrix},$$

from which it is possible to extract a (14×14) minor

$$M = \begin{bmatrix} c^T(\theta) & 0 \\ \vdots & \vdots \\ c^T(\theta)A^6(\theta) & 0 \\ 0 & c^T(\theta) \\ \vdots & \vdots \\ 0 & c^T(\theta)A^6(\theta) \end{bmatrix},$$

that is non singular in Θ, because it is

$$\det\{M\} = [\det\{\mathcal{O}(\theta)\}]^2$$

and $\det\{\mathcal{O}(\theta)\}$ is different to zero, as shown in (40) and commented above.

Similarly the controllability matrix of the couple $(A_T(\theta), B_T(\theta))$ is the (14×28) matrix

$$\mathcal{C}_T(\theta) = \begin{bmatrix} b^{(1)}(\theta) & 0 & A(\theta)b^{(1)}(\theta) & 0 & ... & A^{13}(\theta)b^{(1)}(\theta) & 0 \\ 0 & b^{(2)}(\theta) & 0 & A(\theta)b^{(2)}(\theta) & ... & 0 & A^{13}(\theta)b^{(2)}(\theta) \end{bmatrix},$$

from which it is possible to extract a (14×14) minor

$$N = \begin{bmatrix} b^{(1)}(\theta) & \dots & A^6(\theta)b^{(1)}(\theta) & 0 & \dots & 0 \\ 0 & \dots & 0 & b^{(2)}(\theta) & \dots & A^6(\theta)b^{(2)}(\theta) \end{bmatrix},$$

which is non singular. In fact its determinant is

$$\det\{N\} = \det\{\mathcal{C}^{(1)}(\theta)\} \cdot \det\{\mathcal{C}^{(2)}(\theta)\}$$

where $\det\{\mathcal{C}^{(1)}(\theta)\}$ and $\det\{\mathcal{C}^{(2)}(\theta)\}$ are the determinants of the controllability matrices related, respectively, to the doses d_1, d_2 and they are different from zero, as already shown in (41). So the triple $(A_T(\theta), B_T(\theta), C_T(\theta))$ is observable and controllable.

Now we can prove the following result.

Theorem 5. The model (5) - (7) is globally identifiable with respect to the unknown parameter vector θ given by (50) and ranging in the set Θ defined by (51), exploiting the outputs of the model obtained from two different radiation doses.

Proof. Given $\theta \in \Theta$, let us consider the (1×6) vector

$$\phi = \begin{bmatrix} \chi^* \\ \chi_D^* \\ \mu_D^* \\ \mu_N^* \\ \delta_1^* \\ \delta_2^* \end{bmatrix} \in \Theta$$

and the (14×14) matrix

$$P = \begin{bmatrix} p_{11} & p_{12} & \cdots & p_{114} \\ p_{21} & p_{22} & \cdots & p_{214} \\ \vdots & \vdots & \ddots & \vdots \\ p_{141} & p_{142} & \cdots & p_{146} \end{bmatrix}.$$

As already done in the proof of Theorem 3, let us divide the P matrix into four (7×7) blocks, as in (32). We obtain the same four subsystems (33), where now the triple $(A_T(\theta), B_T(\theta), C_T(\theta))$, is the one defined in (53).

The subsystems (a) and (d) of (33) are similar to the one studied above in this section. So both the subsystems have only the trivial solution (θ, I). So from (a) and (d) it results that

$$\begin{cases} \chi^* = \chi \\ \chi_D^* = \chi_D \\ \mu_D^* = \mu_D \\ \mu_N^* = \mu_N \\ \delta_1^* = \delta_1 \\ \delta_2^* = \delta_2 \\ P_{11} = P_{22} = I \end{cases} . \tag{55}$$

It is simple to verify, as in the proof of Theorem 3, that, with the results (55), the subsystems (c) and (b) of (33) give the solutions $P_{12} = P_{21} = 0$.

Therefore we have that system (16) admits only the trivial solution (θ, I). Thus, from lemma 1, we can say that the model (5) - (7) is globally identifiable by exploiting the model response $y_T(t;\theta)$ to at least two different doses d_1 and d_2.

□

4 Concluding remarks

In this paper we have considered two spatially uniform dynamical models of tumour growth after a single instantaneous radiative treatment: the basic model of Section 2.2 and the model with multiple subcompartments of Section 2.3. In these two models the details of the spatial structure of the tumour are neglected and the attention is focused on the temporal evolution of tumour overall volume after the radiative treatment. The models can be used for different applications. For instance, to asses the efficiency of the radiotherapeutic treatment, but for this application it is necessary to identify the unknown parameters. A preliminary condition, that is necessary to verify before performing the parameter estimation, is the global identifiability of the model.

In this paper a detailed study of the identifiability properties of the two models is done, pointing out that the basic model is only locally identifiable, whereas the model with subcompartments is globally identifiable, provided that the responses to two different radiation doses are considered.

Therefore the model with subcompartments shows better properties with respect to the basic one. In fact, in addition to a significantly better fit that can be obtained by this model with respect to the fitting obtainable with the basic model, it enjoys the important property of global identifiability that assures a correct formulation of the parametric identification problem. The parametric identification of the model and the corresponding validation, with respect to both the fitting and the prediction capability of the experimental data, are treated in [2].

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